GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 10/16/79

Project Title: Synthetic Protease Inhibitors

Project No: G-33-F04 Green card.

Project Director: Dr. J. C. Powers

Sponsor: DHEW/PHS/NIH - National Heart, Lung & Blood Institute
Bethesda, MD 20014

Agreement Period: From 8/31/79 Until 8/30/80 (05 Year)

Type Agreement: Grant #5R01 HL18679-05

Amount: $52,696 New PHS Funds (G-33-F04)
3,357 GIT Contribution (G-33-352)
$56,053 Total

Reports Required: Annual Progress Reports with Continuation Applications
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

| Technical Matters | Contractual Matters (thru OCA) |
|---|---|
| Program Contact:<br>Dr. R. Sohn<br>301/496-7332 | Grants Management Contact:<br>Mr. Roger Deshaies<br>301/496-7255 |
| Program Official:<br>Claude Lenfant, M. D.<br>Director | Grants Management Official:<br>James M. Pike<br>Chief |
| Division of Lung Diseases<br>Nat'l Heart, Lung, & Blood Institute<br>Bethesda, MD 20014 | Grants Operation Branch<br>Division of Extramural Affairs<br>Nat'l Heart, Lung, & Blood Institute<br>Bethesda, MD 20014 |

NOTE: FOLLOW-ON TO PROJECT G-33-F03 (04 YR.)
Defense Priority Rating: none

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director—EES
- Accounting Office
- Procurement Office
- Security Coordinator (OCA)
- Reports Coordinator (OCA)
- Library, Technical Reports Section
- EES Information Office
- EES Reports & Procedures
- Project File (OCA)
- Project Code (GTRI)
- Other C. E. Smith

CA-3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

# SPONSORED PROJECT TERMINATION

Date: August 20, 1981

Project Title: Synthetic Protease Inhibitors

Project No: G-33-F04

Project Director: Dr. J. C. Powers

Sponsor: DHEW/PHS/NIH - National Heart, Lung & Blood Institute

Effective Termination Date: 8/30/80

Clearance of Accounting Charges: -----

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
X Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
_ Other _____

NOTE: Follow-on project (06 year) is G-33-F05

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

Administrative Coordinator
Research Property Management
Accounting Office
Procurement Office
Research Security Services
~~Reports Coordinator (OCA)~~
Legal Services (OCA)
Library, Technical Reports
EES Research Public Relations (2)
Project File (OCA)
Other: _____

G-33-F04

| APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 → | GRANT NUMBER |
|---|---|
| **SECTION IV—SUMMARY PROGRESS REPORT** | HL 18679-06 |
| PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) | PERIOD COVERED BY THIS REPORT |
| Powers, James C. | FROM: 9/1/79 — THROUGH: 6/15/80 |
| NAME OF ORGANIZATION: Georgia Institute of Technology | |

TITLE (Repeat title shown in Item 1 on first page)
Synthetic Protease Inhibitors

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. "Virus-specified Protease in Poliovirus-infected Hela cells", B. Korant, N. Chow, M. Lively and J. C. Powers, Proc. Nat. Acad. Sci., 76, 2992-2995 (1979).
"Inhibition of Thermolysin and Carboxypeptidase A by Phosphoramidates", C. Kam, N. Nishino, and J. C. Powers, Biochemistry, 18, 3032-3038 (1979).
"Design of Potent Reversible Inhibitors for Thermolysin. Peptides Containing Zinc Coordinating Ligands and Their Use in Affinity Chromatography", N. Nishino and J. C. Powers, Biochemistry, 18, 4340-4347 (1979).
The Effect of the Specific Elastase Inhibitor, Alanyl Alanyl Prolyl Alanine Chloromethylketone, on Elastase-Induced Emphysema", J. Kleinerman, V. Ranga, D. Rynbrandt, M. P. C. Ip, J. Sorensen and J. C. Powers, Am. Rev. Respiratory Disease 121, 381-387 (1980).
"A Zinc Metalloendopeptidase Associated with Dog Pancreatic Membranes", R. A. Mumford, A. W. Strauss, J. C. Powers, P. A. Pierzchala, N. Nishino and M. Zimmerman, J. Biol. Chem., 255, 2227-2230 (1980).
"Pseudomonas aeruginosa Elastase. Development of a New Substrate, Inhibitors and an Affinity Ligand", N. Nishino and J. C. Powers, J. Biol. Chem., 255, 3482-3486.

2. No changes.

3. Progress Report.

Overall Objectives for Total Project. A number of proteolytic enzymes such as elastase and collagenase have been shown to be involved in diseases such as pulmonary emphysema and arthritis which involve tissue destruction. The goal of this proposed research is to design and synthesize specific and effective inhibitors for these proteolytic enzymes. The inhibitors should be invaluable in the study of the normal biological function and the role of these proteases in disease. In addition, synthetic protease inhibitors should find use in the clinical treatment of plumonary emphysema, rheumatoid arthritis and other diseases.

Goals for the Current Year. Our goals for the current year were to develop new types of inhibitors for human leukocyte elastase (a serine protease) and to continue work on developing specific metalloprotease inhibitors.

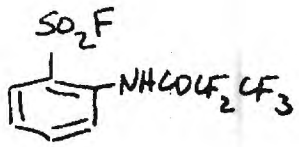
Studies with Human Granulocyte Enzymes. Proteolysis by enzymes released from human PMN leukocytes, macrophages and other sources are involved in several major diseases which involve tissue destruction. In the case of pulmonary emphysema, elastase seems to be principally responsible for lung damage with collagenase, cathepsin G and other proteases carrying out secondary digestions. We have previously synthesized a number of specific peptide chloromethyl ketone inhibitors of both human leukocyte elastase and cathepsin G. Three of the best elastase inhibitors, MeO-Suc-Ala-Ala-Pro-Val$CH_2Cl$, Suc-Ala-Ala-Pro-Val$CH_2Cl$ and Ac-Ala-Ala-Pro-Ala$CH_2Cl$, have been shown to be effective at preventing emphysema by three research groups (Dr. Aaron Janoff, SUNY Stoney Brook; Dr. P. Stone, Boston University; and Dr. J. Kleinerman, Mt. Sinai Medical Center). In all cases the hamster emphysema animal model was utilized. Even though our peptide chloromethyl ketones are effective, there has been considerable concern about their toxicity. Chloromethyl

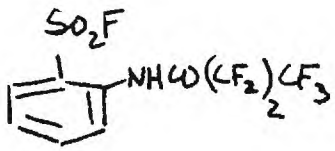


PHS-2590-1 (Formerly NIH-2006-1)
(Rev. 4-75)
☆U.S. GOVERNMENT PRINTING OFFICE: 1979 O— 281-217/3055

ketones are alkylating agents and would be expected to exhibit some carcinogencity. Thus most investigators believe that these compounds may not have utility for the treatment of human disease. Therefore, we have begun a search for specific elastase inhibitors which have properties which would allow their utilization in humans.

We have been investigating sulfonyl fluorides as elastase inhibitors and have discovered several excellent inhibitors of human leukocyte (HL) elastase.



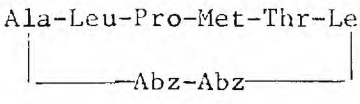


| | | | |
|---|---|---|---|
| HL elastase | 1700 | 1400 | 1200 |
| PP elastase | 1300 | 62 | 26 |
| cathepsin G | 13 | 5.8 | 13 |

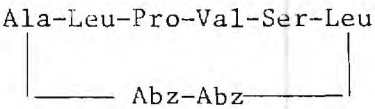
The values listed under each compound are inhibition rates ($k_{obs}/[I]$). These compounds are simply representatives of many sulfonyl fluorides which we have synthesized and tested. All three are very effective inhibitors of human leukocyte elastase and the latter two are quite specific. Sulfonyl fluorides are not generally considered to be toxic and thus elastase inhibitors such as these may find utility in the treatment of human disease. P. Stone and J. Kleinerman have agreed to test a sulfonyl fluoride in their animal models of emphysema.

Our goals for next year are to improve the reactivity and specificity of this class of compounds. Many of the sulfonyl fluorides hydrolyze quite rapidly in water and we plan to synthesize analogues which may exhibit greater stability toward hydrolysis.

Small Peptide Analogs of the $\alpha_1$-Protease Inhibitor. Another approach to elastase inhibitors is to make analogs of the $\alpha$-protease inhibitor ($\alpha_1$-antitrypsin) active site. The sequence at the active site has recently been determined by Dr. J. Travis at the U. of GA. We were then able to design and synthesize small cyclic peptides such as 1 which have the $\alpha_1$-PI active site sequence. This peptide is a reversible inhibitor of human leukocyte elastase ($K_I$ = 0.38mM) and is not a substrate. We then replaced the Met-Thr




1




2

Abz = 3-aminobenzoyl

residues with Val-Ser to get 2 which was a poorer inhibitor ($K_I$ = 0.80mM). However, a minor by-product of the reaction was a 20-fold better inhibitor ($K_I$ = 0.040mM). At present, we are uncertain as to the structure of the by-product, but suspect racemization at the Ala residue.

Although the cyclic peptides are only moderate inhibitors at present, they are good lead compounds for the development of better structures. Our first goal for the next year is to determine the structure of the by-product which is the best HL elastase of the series. Cyclic peptide analogs of $\alpha_1$-PI are likely to be not toxic due to their

close resemblance to the natural inhibitor. Synthetic elastase inhibitors seem to offer the best hope at present for the treatment of the majority of emphysema since natural $\alpha_1$-PI is difficult to isolate and purify.

Studies with Metalloproteases. A number of metalloproteases are involved in diseases which involve connective tissue destruction. Collagenase has been found in rheumatoid synovium and has been implicated in the destruction of joints in rheumatoid arthritis. Collagenase may also be involved in periodontal disease, corneal ulceration, and several other diseases. Invasive tumors have been shown to secrete collagenase and the ability of this enzyme to attack connective tissue may allow such tumors to expand into the surrounding tissue.

Excellent progress has been made in the development of general classes of inhibitors for the metalloproteases family. Using thermolysin and carboxypeptidase as model systems in our initial experiments, we have investigated phosphoramidates, hydroxamic acids as irreversible inhibitors. Phosphoramidates such as P-Leu-$NH_2$ and P-Phe-$O^-K^+$ are excellent inhibitors of thermolysin and carboxypeptidase A respectively. The hydroxamic acid NONH-Bzm-Ala-Gly-$NH_2$ (Bzm = $-COCH(CH_2C_6H_5)CO-$) is a specific inhibitor of thermolysin ($K_I$ = 0.7 μM) and has been attached to agarose and used in the affinity purification of thermolysin. A number of irreversible thermolysin inhibitors such as $ClCH_2CON(OH)CH(CH_2CH(CH_3)_2CO_2CH_3$ have been designed and synthesized. The site reaction has been determined. Several hydroxamic acids thiols, and phosphoramidates with the appropriate sequence to inhibit collagenase have been synthesized. Some were observed to be moderate inhibitors.

Pseudomonas aeruginosa elastase is an infectious organism which is resistant to many antibiotics. This organism causes hemorrhagic pneumonia in mink and corneal ulcers in man. The major cause of morbidity and mortality in cystic fibrosis is the severe, chronic presistent pulmonary infection with bacteria particularily P. aeruginosa. Many strains of P. aeruginosa produce an elastase. Those strains with elastase have been shown to be more pathogenic than those without. The elastase is likely the factor responsible for the destruction of corneal tissue and hemorrhages of the lung observed in P. aeruginosa infections.

P. aeruginosa is a zinc metalloprotease and we have developed a new substrate to assay the enzyme. Specific inhibitors for this elastase have been designed and synthesized. In particular, the hydroxamic acid $HONH-COCH_2CH(CH_2C_6H_5)CO$-Ala-Gly-$NH_2$ was a potent reversible inhibitor ($K_I$ = 0.044 μM) and $ClCH_2CO$-HO-Leu-Ala-Gly-$NH_2$ was an irreversible inhibitor. Both compounds may find utility in the treatment of infections due to P. aeruginosa elastase.

Our goals for next year are to extend inhibitors to collagenase and improve the inhibitor for P.a. elastase. In particular, a more effective irreversible inhibitor is needed and we plan to synthesize and test compounds such as $CH_2$=CH-CO-HOLeu-Ala-Gly-$NH_2$.

Significance. It is the belief of the author that reagents which control the activity of proteolytic enzymes can be used in a number of clinical situations. Diseases involving tissue destruction such as emphysema and arthritis have been shown to involve enzymes such as elastase, cathepsin G and collagenase. Invasive tumors secrete collagenase, possibly accounting for their ability to expand into the surrounding connective tissue. Viral protein processing requires a protease. Organisms like Neisseria Gonorrhoeae and N. meningitidis secrete proteases which cleave the principal mucosal antibody, immunoglobulin A., and P. aeruginosa produces an enzyme which destroys lung tissue.

The basic goal of our research is to develop new classes of inhibitors for the two major families of proteases: serine and metalloproteases. Within this framework our emphasis have been directed toward inhibitors for granulocyte elastase and cathepsin G, and collagenase since these enzymes are involved in two major chronic diseases: emphysema and arthritis. In the course of this work we are learning new information about these

specific enzymes and about the two general classes of proteases. In addition, we are discovering ways to increase the specificity of inhibitors both for a specific enzyme within a class of proteases and for an enzyme when it is located in its natural enviroment which may contain a multitude of other reactive groups. The information should be useful to other investigators who desire specific inhibitors for other proteases.

At present some of our elastase inhibitors are being tested in animals for the treatment of emphysema. In fact, we are currently synthesizing 9 g of a chloromethyl ketone elastase inhibitor in order for J. Kleinerman (Mt. Sinai Med. Center) to carry out a 6-month study of the effect of daily doses of the inhibitor on the progression of emphysema in hamsters. There is a good possibility that the course of emphysema can be arrested by use of the appropriate inhibitor. At present, better elastase inhibitors are desired. Our studies with synthetic protease inhibitors are leading us closer to clinically useful drugs.

The undersigned agrees to accept responsibility for the scientific technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

6/18/80
Date

James C. Powers
Principal Investigator or Progam Director